Exhibit 1

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Different mRNA and Protein Expression of Matrix Metalloproteinases 2 and 9 and Tissue Inhibitor of Metalloproteinases 1 in Benign and Malignant Prostate Tissue

Ralf Lichtinghagen[a], Petra B. Musholt[a], Michael Lein[b], Andreas Römer[b], Birgit Rudolph[c], Glen Kristiansen[c], Steffen Hauptmann[c], Dietmar Schnorr[b], Stefan A. Loening[b], Klaus Jung[b,*]

[a]*Department of Clinical Chemistry, University Medical School, Hannover, Germany*
[b]*Department of Urology, University Hospital Charité, Humboldt University Berlin, Schumannstraße 20/21, D-10098 Berlin, Germany*
[c]*Department of Pathology, University Hospital Charité, Humboldt University, Berlin, Germany*

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Abstract

Objective: The aim of this study was to assess the behavior of the matrix metalloproteinases (MMPs) 2 and 9 and the tissue inhibitor of metalloproteinases 1 (TIMP-1) in human prostate cancer.

Methods: mRNA and protein expression patterns of MMP-2, MMP-9, and TIMP-1 were studied in cancerous and noncancerous parts of 17 prostates removed by radical prostatectomy. Competitive RT-PCR, gelatin-substrate zymography, and ELISA techniques were used for quantification.

Results: On the mRNA level, MMP-2 expression was decreased and MMP-9, TIMP-1, the ratios of MMP-2 and MMP-9 to TIMP-1 were unchanged in cancerous tissue compared to the normal counterparts. On the protein level, expression of MMP-9 was significantly higher and TIMP-1 expression was significantly lower, MMP-2 was unchanged and the ratios of MMP-2 and MMP-9 to TIMP-1 were increased in tumor tissue.

Conclusions: The higher concentration of MMP-9 as well as the increased ratios of MMP-2 and MMP-9 to TIMP-1 in malignant tissue prove the proteolytic dysbalance in prostate cancer, which does not seem to be associated with the stage and grade of the tumor. Comparison of mRNA and protein expression of MMP-2, MMP-9 and TIMP-1, respectively, did not show any significant relationships illustrating the necessity to study these components at both molecular levels.

Keywords: Prostate cancer; Tumor marker; Matrix metalloproteinases; Metastasis; Expression profile

1. Introduction

Matrix metalloproteinases (MMPs) belong to a growing family of proteases sharing the ability to degrade extracellular matrix components and to destroy the basement membrane [1]. Both in vitro and in vivo investigations performed over the past few years have demonstrated their important role in tumor invasion and metastasis [2].

Studies on MMPs and TIMPs have been performed in prostate tumors grown in animals [3,4], in malignant prostatic cells [5–7], and in cell cultures of human prostatic tissue [8–11]. Increased MMP-2 and MMP-9 but reduced TIMP-1 concentrations were found in conditioned media of epithelial cultures from neoplastic prostate [8]. However, there are sparse data characterizing the corresponding state in human prostatic tissue directly [12–14]. These results, mostly based on immunohistochemical and in situ hybridization data, have the disadvantage not to be of truly quantitative nature. Although it is well known that MMPs are controlled at several steps—including gene transcription, transfor-

* Correspondence author. Tel. +49-30-450-515041; Fax: +49-30-450-515904.
E-mail address: klaus.jung@charite.de (K. Jung).

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mation of latent enzymes into active forms, and inhibition by tissue inhibitors [2]—comparative data of MMP expression in prostate carcinoma tissue on both the mRNA level and protein level are still lacking.

To get further insights into this problem, the aims of this study were (1) to evaluate the expression patterns of MMP-2, MMP-9, and TIMP-1 in benign and malignant prostatic tissue; (2) to compare the expression of these matrix components on different molecular levels, e.g. the mRNA and protein level; (3) to determine the association of MMPs and TIMP-1 with tumor stage and grade.

2. Materials and methods

2.1. Patients and tissue samples

The patient cohort consisted of 17 white patients (mean age, 64 years; range from 56 to 68 years) who had undergone radical retropubic prostatectomy for prostatic adenocarcinoma. The patients were untreated before surgery with prostate-specific antigen concentrations of 9.1 ± 4.3 µg/l. The cancer stage was assigned according to the TNM system; the histological grade was categorized as grade 1, 2 and 3 as well as according to the Gleason score [15]. The pathological stages and grades of the 17 patients were as follows: pT2pN0M0 ($n = 10$), pT3pN0M0 ($n = 7$); G2 ($n = 12$), G3 ($n = 5$); 15 patients had Gleason scores between 3 and 6, and two subjects had Gleason scores of 7 or greater. Five tumors were classified as having positive surgical margins (R1) and 12 tumors were organ-confined (R0).

The use of tissue for research purposes was approved by the Ethic Committee of the Hospital. Surgical specimens obtained at radical prostatectomy were sectioned and pathologically evaluated according to diagnostic demands. Fresh prostate tissue samples were obtained from the cancerous and noncancerous parts of the same prostate. These matched pairs were used for further analysis. The investigation of matched pairs of the same organ has been considered the best way to compensate interindividual differences. Small pieces of tissue were dissected immediately after removal of the prostate by an experienced pathologist (Glen Kristiansen), snap frozen, and stored in liquid nitrogen until analysis. Histological analysis from all tissue pieces utilized was performed as previously described in order to ensure that the material consisted of either malignant or benign tissue [16]. Only tumor samples that were fully surrounded by malignant tissue according to this analysis were used in this study. We also did not include any samples in which benign prostate glands made up >10% of total mass. In this way, contamination of the tumor samples with benign glands was minimized. Most of the tumors were located dorso-laterally in the peripheral zone of the prostate. The tissue characterized as "normal" was usually taken from the inner zone of the contralateral lobe.

2.2. mRNA isolation and protein extraction

About 25–35 mg of tissue was homogenized in 400 µl DNA/RNA Stabilization Reagent for Blood/Bone Marrow® (Roche Diagnostics, Mannheim, Germany) utilizing a Polytron PT 3000 (Kinematica, Littau, Switzerland). The extraction of mRNA was performed employing a standard protocol (mRNA Isolation Kit for Blood/Bone Marrow®, Roche Diagnostics). RNA samples were tested on agarose gel for degradation and possible contamination with residual genomic DNA. RNA quantification was performed using RiboGreen® RNA Quantitation Kit (MoBiTec, Goettingen, Germany).

To determine MMPs and TIMP-1 by zymography and ELISA technique, tissue extracts were prepared by a combined extraction with 0.25% Triton-X100/10 mM $CaCl_2$ and heat at 60 °C [17]. Protein concentrations were measured with Coomassie brilliant blue assay reagent (Bio-Rad, München, Germany).

2.3. Analysis of the mRNA expression of MMPs and TIMP-1

Competitive RT-PCR for MMP-2, MMP-9, and TIMP-1, respectively, was performed using the Titan One Tube RT-PCR System® (Roche Diagnostics) as described previously [18]. Additional primers (upstream, downstream) were employed for the amplification of the housekeeping gene GAPDH: (5′-TTC ACC ACC ATG GAG AAG GCT G-3′, 5′-CTT CCA CGA TAC CAA AGT TGT C-3′).

In brief, fixed amounts of in vitro transcribed competitor RNA were added to each assay. The same primer pairs bind to cDNA of competitor RNA and to cDNA of natural mRNA origin, respectively, but differences in the sequences of both cDNAs enable hybridization with different capture probes for ELISA detection. In order to generate these RNA standards, the cDNA regions from nucleotides 1279 to 1665 of MMP-2, 1319 to 1938 of MMP-9, 387 to 710 of TIMP-1, and 357 to 583 of GAPDH cDNA were cloned into bacterial transcription vectors [19]. The cloning strategy was published elsewhere [20]. For the construction of competitors, several base substitutions were introduced into the capture probe binding region as described earlier [18].

For the purpose of validation and calibration of the procedure, varying amounts of in vitro transcribed RNA (10^3 to 5×10^9 molecules) equal to mRNA for MMP-2, MMP-9, TIMP-1, and GAPDH were co-amplified with competitor RNA: 10^5 molecules competitor RNA for quantification of MMP-2 and TIMP-1, respectively; 10^6 molecules for MMP-9; and 5×10^9 molecules for the quantification of GAPDH. Concentration of in vitro transcribed RNA was determined photometrically. For labeling of PCR products, digoxigenin-11-dUTP (6 µM final concentration; Roche Diagnostics) was added to be incorporated during amplification.

For the quantification of amplified cDNA, an ELISA procedure (Enzymun Test®, Roche Diagnostics) was performed on an automated ES-300 analyzer (Roche Diagnostics) using different biotinylated internal oligonucleotides for hybridization with amplified cDNA of either mRNA originating from the sample analyzed or synthetic competitor RNA as described previously for MMP-9 and TIMP-1 [18]. Additional 5′-biotinylated capture probes were used for quantification of MMP-2 and GAPDH were as follows: MMP-2 probe: 5′-CAC ATT CTG GCC TGA GCT CC-3′; MMP-2 competitor probe: 5′-CTG TGG ATC CCC TGA GCT CC-3′; GAPDH probe: 5′-CAT CAG CAA TGC CTC CTG CA-3′; GAPDH competitor probe: 5′-CAT CAG CTT AGG ATC CTG CA-3′.

This procedure of separate hybridization to template cDNA and synthetic competitor cDNA with different capture probes yields signals equivalent to the amount of cDNA of sample mRNA origin and competitor RNA origin. Amplification of varying amounts of template in combination with a fixed amount of a competitor yields a linear curve in a double-logarithmic plot expressing the amount of a varying template on one scale and the ratio of both PCR products (template/competitor) on the other scale. The number of molecules per nanogram mRNA were retrieved from calibration curves generated with fixed amounts of in vitro transcribed standard cDNA resembling the template sequences. Data for each calibration curve were calculated using linear regression. One calibration curve was performed for each run.

2.4. Analysis of the protein expression of MMPs and TIMP-1

Zymography for MMP-2 and MMP-9 was performed in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 8%) containing 0.5% gelatin on the Midget Electrophoresis Unit 2050 (LKB, Uppsala, Sweden). Equal protein amounts extracts (12 µg; 3–6 µl) and at least two samples of human proMMP-2 and proMMP-9 (Chemicon Inc., Temecula, USA) representing standards of the linear part of the calibration curve were included in each gel run. After electrophoresis, gels were soaked for 2 h in 2.5% Triton-X 100 solution with four washing steps. The gels were then incubated for 18 h at 37 °C in buffer containing 50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 10 mM $CaCl_2$, 0.2% Brij-35, and 0.02% NaN_3. After incubation, the gels were stained with 0.2% Coomassie blue and destained until clear proteolytic bands appeared. Gels were scanned using a flatbed scanner (Scanmaker 4; Microtek Lab, Redondo Beach, USA). The band intensities were determined by the software Scion Image (Scion Corp., Frederick, MD, USA). The amount of MMPs loaded was calculated using the calibration curve. Studies with inhibitors (EDTA; *o*-phenanthroline) and activators (*p*-amino-phenylmercuric acetate) verified the specificity of the method.

TIMP-1 was measured by the BIOTRAK™ ELISA kit (RPN 2611, Amersham Pharmacia Biotech UK Ltd., Little Chalfont, UK) as previously described [17]. MMP-2 and MMP-9 were also determined using the BIOTRAK™ chromogenic immunocapture assays (RPN 2630 and 2631, Amersham). The difference between the values with and without the addition of *p*-amino-phenylmercuric acetate corresponds to the proforms of MMP-2 and MMP-9.

2.5. Statistical analysis

Data were analyzed using the statistical software packages SPSS 10.0 for Windows (SPSS, Chicago, USA). Wilcoxon's signed rank test and Student's *t*-test for paired data, the calculation of rank correlation coefficients according to Spearman, and logistic regressions were performed. Differences were considered to be significant at $p < 0.05$.

3. Results

The reliability of the measurements was proven by analytical controls (integrity test of RNA; between-run controls of RT-PCR and zymography with coefficients of variation <15%). In addition, the cancerous and noncancerous samples were always measured in one run to reduce the variability between the paired samples.

Gelatin zymograms reveal two remarkable findings (Fig. 1): (a) the proforms of MMP-2 and MMP-9 are the major forms in prostatic tissue while activated MMP forms corresponding to bands below the main bands of the proforms were not detected; (b) the patterns showed higher MMP-9 levels in cancerous than in noncancerous parts of the prostate.

The evaluation of all these data is shown in the following Figs. 2 and 3. Scatter plots of the mRNA expressions of MMP-2, MMP-9, TIMP-1, their ratios, and the house-keeping gene GAPDH showed no statistical differences ($p > 0.05$) between the paired cancerous and noncancerous samples except for the decreased mRNA MMP-2 values ($p = 0.002$) (Fig. 2). GAPDH mRNA expression was not different between matched pairs of normal and cancerous samples. Thus, a correction of data with house-keeping gene expression values was not done and did not improve the significance of our data. Fig. 3 shows the corresponding protein data obtained by gelatin zymography for MMPs and by ELISA for TIMP-1. MMP-2 and MMP-9 were also partly measured using the BIOTRAK ELISA with and without *p*-aminophenylmercuric acetate. Both MMPs were not detectable without activation by *p*-aminophenylmercuric acetate suggesting that MMP-2 and MMP-9 mainly occur as proMMP forms in prostatic tissue. These results support zymographic data mentioned above (Fig. 1). While MMP-2 values (Fig. 3A) did not differ ($p > 0.05$) between cancerous and noncancerous samples, MMP-9 values (Fig. 3B) were significantly higher ($p = 0.042$) and values of TIMP-1 (Fig. 3C) were significantly lower ($p = 0.023$) in malignant versus benign tissue. Both ratios MMP-2/TIMP-1 and MMP-9/TIMP-1 were significantly increased (Fig. 3D and E).

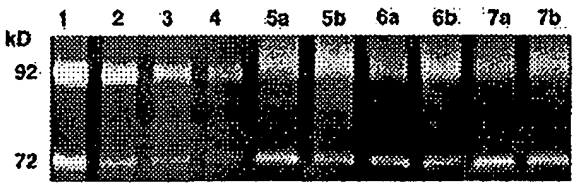

Fig. 1. Gelatin zymography of standards of MMP-2 and MMP-9 and of protein extracts of noncancerous and cancerous tissue samples of the same prostate obtained at radical prostatectomy. Lanes 1–4 contained standards of MMP-2 (72 kD) and of MMP-9 (92 kD): 12, 9.6, 6.0, 4.8 ng of purified proMMP-2 and 4.8, 4.0, 2.4, 1.7 ng of purified proMMP-9, respectively, (further details see Section 2) were loaded to make calibration curves. Lanes 5–7: typical digestion patterns of the extracts from matched pairs of noncancerous ("a" behind the number) and cancerous ("b" behind the number) parts of three prostates obtained at radical prostatectomy. Equal protein amounts (12 µg) were loaded. MMP-9 were generally higher in cancerous than in noncancerous samples.

There were no significant correlations between the mRNA expression and the protein expression of MMP-2, MMP-9, and TIMP-1, respectively, both for the combined set of cancerous and noncancerous pairs (Fig. 4A–C) and for the separate benign and malignant samples (data not shown). On the mRNA level, a significant correlation between the expression rates was observed for MMP-2 and TIMP-1 only ($r_s = 0.66$, $p < 0.001$), whereas there was an almost significant correlation of $r_s = 0.43$ ($p = 0.06$) between the expression of MMP-2 and MMP-9 on the protein level.

We also used the logistic regression analysis and the discriminant analysis to predict prostate cancer, utilizing MMP-2, MMP-9, and TIMP-1 on the mRNA and

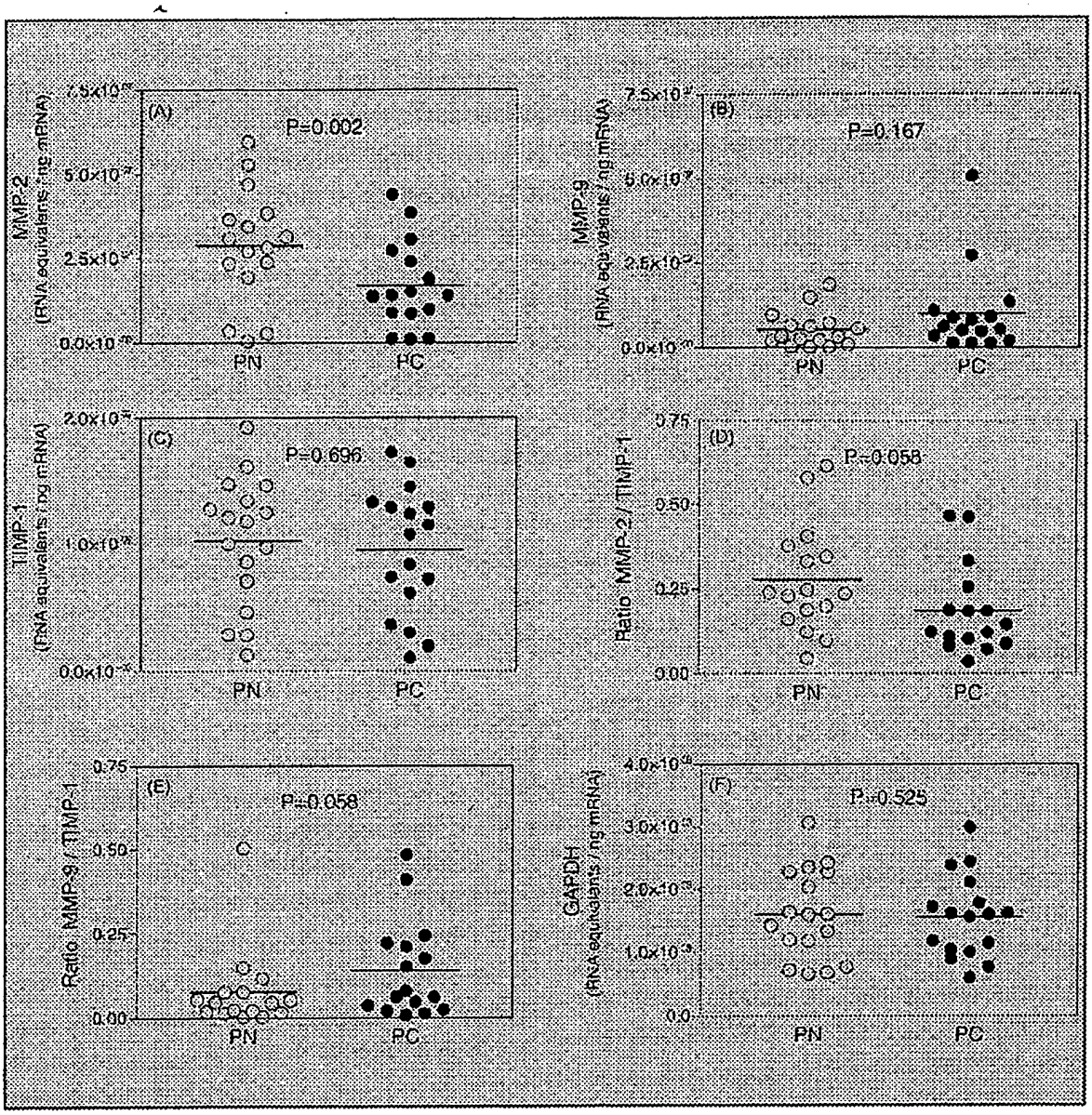

Fig. 2. Scatter plots of (A) MMP-2, (B) MMP-9, (C) TIMP-1, and the ratios of (D) MMP-2/TIMP-1 and (E) MMP-9/TIMP-1 measured as mRNA expression by quantitative RT-PCR in (PN) noncancerous and (PC) cancerous tissue samples of the same prostate obtained after radical prostatectomy. Horizontal lines indicate mean values. Statistical differences between the malignant and benign tissue samples are indicated as *p*-values for paired data.

protein level. With a stepwise selection method, the ratio of MMP-9 to TIMP-1 on the protein level and MMP-2 on the mRNA level, respectively, were the best indicators with the overall correct classification of only 70–80%.

The correlation matrix between clinical data (tumor staging, grading, prostate-specific antigen) and the tissue MMP parameters did not result in any remarkable associations except for the association of tumor stage and grade with the expression of MMP-2 (Table 1). However, it should be mentioned that only two tumors had Gleason score of 7 or greater so that the correlation data did not include the possible effect of more aggressive tumors.

4. Discussion

Our results prove the disparities of MMP-2, MMP-9, and TIMP-1 expressions in cancerous and noncancer-

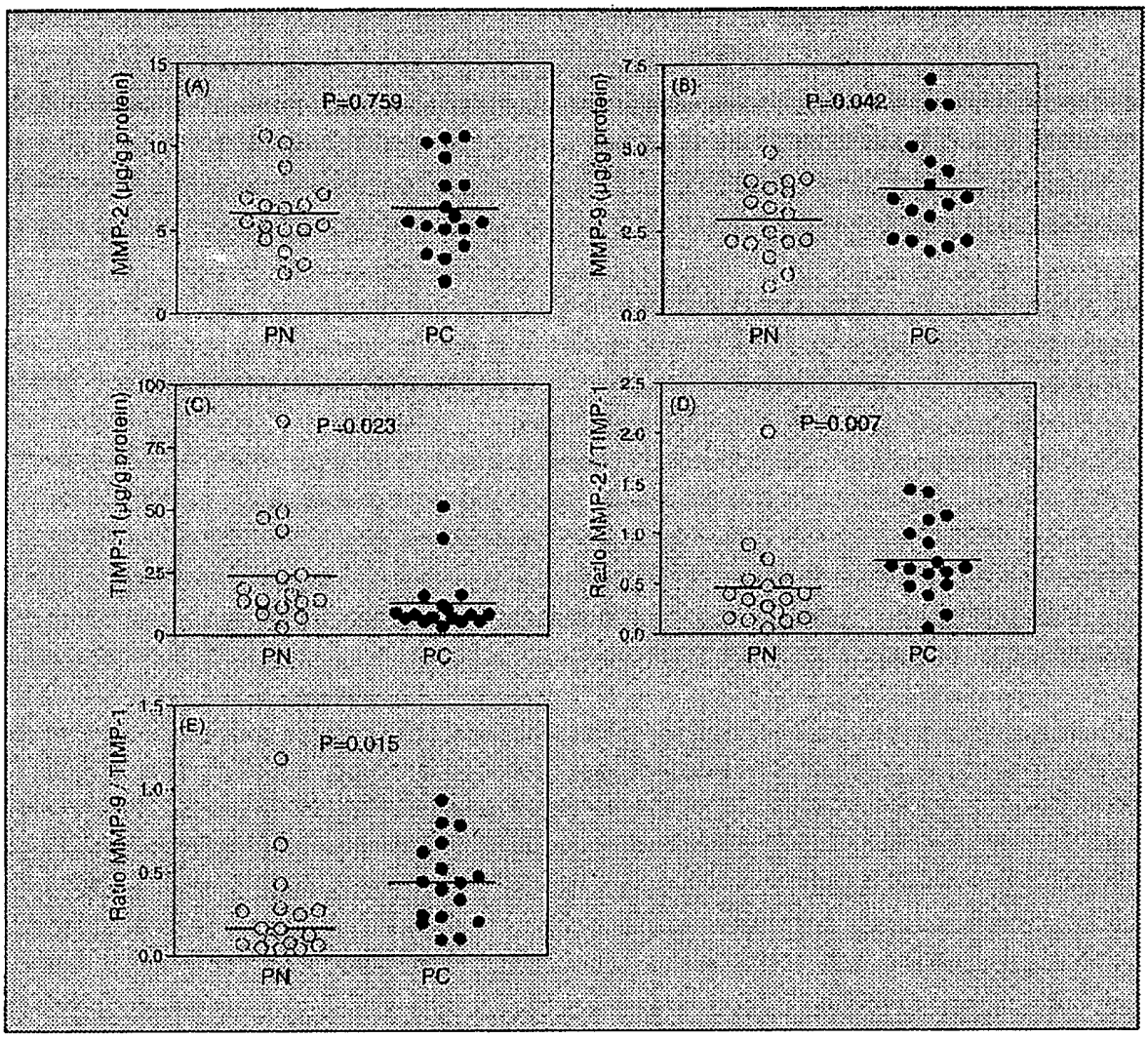

Fig. 3. Scatter plots of (A) MMP-2, (B) MMP-9, (C) TIMP-1, and the ratios of (D) MMP-2/TIMP-1 and (E) MMP-9/TIMP-1 measured as protein expression by zymography and ELISA in (PN) noncancerous and (PC) cancerous tissue samples of the same prostate obtained after radical prostatectomy. The concentrations analyzed were related to the total tissue protein. Horizontal lines indicate mean values. Statistical differences between the malignant and benign tissue samples are indicated as p-values for paired data.

ous prostatic tissue in several aspects. First, the expressions on the mRNA and protein levels were obviously non-coordinated and different (Fig. 4). Second, their expression rates were neither correlated at the mRNA (except for associations between MMP-2 and TIMP-1) nor at the protein level. Third, the expression rates and ratios of MMPs to TIMP-1 were partly different between benign and malignant prostatic tissue showing increased expression of MMP-9 and increased ratios of MMP-2 and MMP-9 to TIMP-1, respectively, on the protein level. Thus, these data are interesting with regard to the molecular and clinical significance of MMPs in prostate cancer.

MMPs have been implicated in the molecular basis of metastasis [1]. For the metastatic process, tumor cells must cross the basement membrane. The main component of the basement membrane is type IV collagen. Especially the type IV collagenases MMP-2 and MMP-9 degrading this component are important in the process of invasion and metastasis. The proteolytic degradation of the basement membrane and extracellular matrix is hypothesized as an important step in metastasis formation [1]. New findings additionally support the view that MMPs also regulate the growth of the tumor by maintaining the access to growth factors from the extracellular matrix and by the regulation of angiogenesis [21].

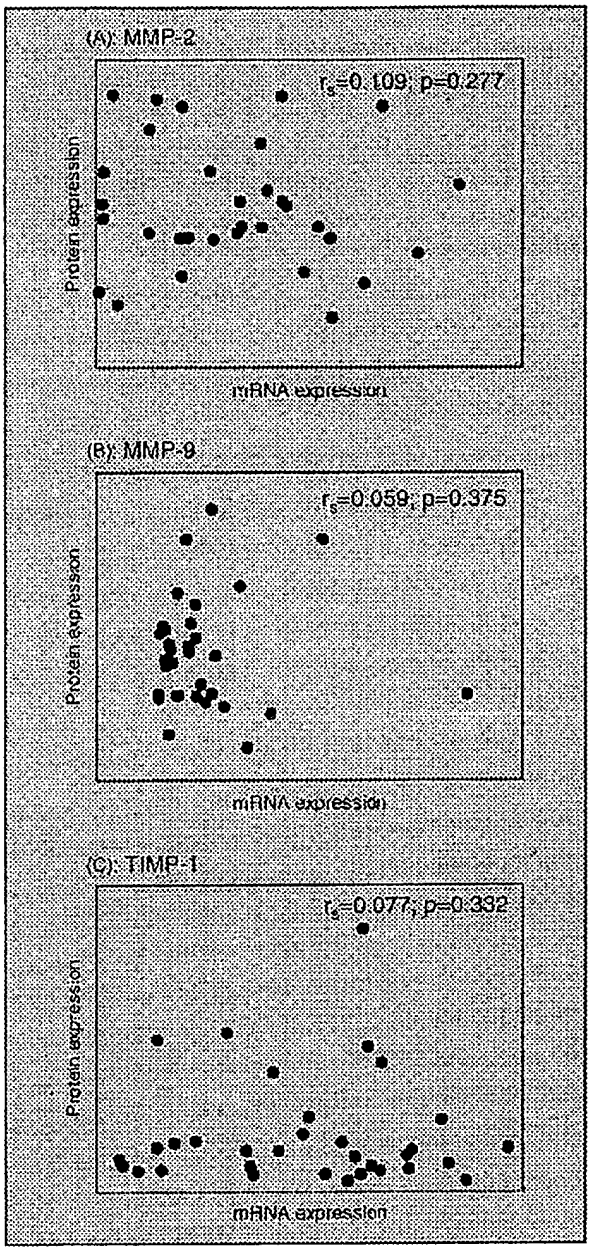

Fig. 4. Correlation matrix between the mRNA expression and protein expression for (A) MMP-2, (B) MMP-9, and (C) TIMP-1 in human prostatic tissue. Spearman rank correlation coefficients were calculated from 34 tissue samples. The corresponding significance levels are indicated for each correlation.

The altered expression pattern of MMPs and TIMPs was demonstrated in various human tumors like breast, colon, and lung cancers [8,22,23]. As mentioned in the Introduction, the significance of MMPs in prostate cancer has been demonstrated in cell culture studies and animal experiments. Only a few studies have been performed in human prostate tissue samples both for MMP-2, MMP-9, and TIMPs [10,13,14,24–28]. Different techniques, such as Northern blot analysis, zymography after electrophoresis, immunohistochemistry, and ELISA techniques have been used. As these techniques reflect the expression either on transcription or on the translation level, differences in this respect may explain the findings. In addition, quantitative RT-PCR as used in our study has not been applied so far. All these techniques show different analytical sensitivities so that different expression patterns could also reflect the higher or lower sensitivity of the procedures applied. To obtain quantitative data of MMPs on the protein level, we utilized the densitometric zymography instead of the conventional immunohistochemistry. A relatively large amount of about 30 mg of tissue specimen was used for protein extraction and the following zymography. Thus, the zymograhic result could be considered representative for the corresponding cancerous or non-cancerous tissue despite a partially heterogeneous distribution of MMPs [11,26,27]. The zymographic technique also facilitates the distinction between the latent and active MMP forms, while the immunohistochemistry has the advantage to demonstrate distribution and localization pattern of the MMPs.

Immunohistochemical data showed increased MMP-2 expression in cancerous prostatic tissue [24–26]. In situ hybridization confirmed the localization in the tumor epithelial cells [27,28], while a relationship between tumor grading and the overexpression was not always found [25]. Increased active, but decreased latent MMP-2 was described in prostate cancer of high Gleason score [13]. Heterogeneous MMP-2 immunostaining with regard to intensity, uniformity, and location was observed [26,27]. For example, more intensely stained cancer cells were found in the cell layer adjacent to the stroma, while cribriform and solid carcinomas showed a more intense staining than acinar carcinomas [26]. Although immunohistochemical and ELISA data were correlated with the mRNA expression rates [24], Northern blot analyses showed lower MMP-2 mRNA expression in tumor grade 1 and 2 were lower in comparison to expression observed in tissue from benign prostatic hyperplasia [28]. These results corresponded to the mRNA MMP-2 data in the present study whereas our quantitative zymographic data opposed these mRNA data. Another study found most of the cancerous tissue samples and none of the normal samples positive for MMP-2 mRNA [14,29].

Increased MMP-9 expression has also been shown in a few studies regarding human prostatic cell cultures and tissue samples [9,10,12,14]. However, the MMP-9

Table 1
Correlations of the tissue concentrations of MMP-2, MMP-9, TIMP-1, and the ratios of MMP-2 and MMP-9 to TIMP-1 with tumor stage, grade, and serum prostate-specific antigen

	Stage	Grade	Prostate-specific antigen
MMP-2 (μg/g protein)	0.708**	0.512*	0.439†
MMP-9 (μg/g protein)	0.268	0.320	0.223
TIMP-1 (μg/g protein)	0.171	0.093	0.181
Ratio MMP-2/TIMP-1	0.244	0.156	0.032
Ratio MMP-9/TIMP-1	0.049	0.087	0.005

The values measured in the cancerous tissue samples were used for correlation analysis. Data presented are Spearman rank coefficients. Staging according to the TNM system. All patients were N0 and M0. Grading according to the Gleason score.
† $p < 0.1$, significance level.
* $p < 0.05$, significance level.
** $p < 0.01$, significance level.

immunostaining was similarly found to be heterogeneous in intensity and location [11]. A strong immunostaining was generally found in highly anaplastic lesions with disorganized glandular structures [11]. MMP-9 is localized in epithelial tumor cells of the prostate rather than in the stromal tissue surrounding the tumor [8,14]. Our data also showed an increased MMP-9 expression on the protein level but not on the mRNA level.

However, our finding of the increased ratios of MMP-9 and MMP-2 to TIMP-1 were as most important. We observed this increased ratio on the protein level but not on the mRNA level. A similar behavior was found when the total MMP activity was measured by a fluorometric enzyme assay [17]. The ratio in favor of the MMPs has been interpreted as an imbalance between the positive and negative factors responsible for a proteolytic equilibrium [1]. It is suggested that this imbalance is more important than the concentrations of the single components of MMPs and TIMPs and may be an essential factor in tumor progression, also for prostate carcinoma [8]. Depending on the tumor, both increased and decreased TIMP-1 concentrations may be possible. For example, in colorectal cancer, increased mRNA TIMP-1 levels were correlated with metastasis, whereas decreased levels were found in pancreatic cancer [30]. These data may indicate that TIMP-1 has opposite effects in regard to tumor progression. Growth-promoting effects were found with TIMP concentrations between 10 and 100 ng/ml, whereas the inhibition of proteolytic degradation of the extracellular matrix needs concentrations <1 μg/ml [31]. Decreased TIMP-1 and TIMP-2 levels were described in prostate cancer depending on the tumor grade (Gleason sum) [14]. Whether the tumor cells themselves or the stroma surrounding the tumor cause this change is still controversial as there are conflicting data on cell localization of TIMPs [14,28]. However, recent cell culture experiments of co-cultures of prostate cancer cells with stromal cells showed that the increased expression of proMMP-9 in prostate cancer cells and the down-regulated expression of TIMPs in stromal cells is a result of differential regulation of these components mediated by a soluble factor in the conditioned medium [32]. These data underline that the tumor-stromal interaction is directly responsible for the opposite behavior of MMPs and TIMPs and could be important for the progression of prostate cancer. The decreased TIMP levels—and in consequence—the increased ratios of MMPs to TIMPs implicate the rationale to use synthetic inhibitors of MMPs in the treatment of prostate cancer.

Our data of the expression of MMPs and TIMP-1 on mRNA and protein levels were partly divergent. In contrast to other authors, we subjected matched pairs of benign and malignant tissue samples of the same prostate to paired comparison analysis and used quantitative assays on the transcriptional and translational/post-translational levels. These quantitative assays, especially the quantitative RT-PCR, are in favor of the more qualitative procedures (e.g. immunohistochemistry, Northern blotting). However, genuine biological reasons, namely, the potential disparities between protein expression and mRNA levels rather than divergent methodologies may explain these differences. Because of the general assumption that mRNA expression is equivalent with a subsequent translation of mRNA to the corresponding protein, little attention has been paid so far to the valid relationship between protein expression and mRNA levels [33]. Both congruent as well as incongruent expression of MMP mRNA or TIMP mRNA was reported in comparison to the corresponding proteins using different techniques and studying various tissues [34–36]. As growth factors are involved in the progression of prostate cancer and differ in their concentrations in cancerous and noncancerous prostate tissue, the expression rates of MMPs and TIMPs may be differently influenced at mRNA and protein levels under physiological and pathological conditions. For example, two non-transcriptional pathways, increased stabilities of both mRNA and extracellular secreted proenzyme, were recently identified as regulatory mechanisms of MMP-2 and MMP-9 influenced by the transforming growth factor beta 1 [36].

Overall, our data of increased ratios of MMP-2 and MMP-9 to TIMP-1 in malignant compared to benign

prostatic tissue prove the proteolytic dysbalance in prostate cancer. This dysbalance does not seem to be associated with the stage and grade of the tumor, but this statement should be verified with a larger sample size. However, the data of this study warrant further experiments applying synthetic inhibitors of MMPs in prostate cancer research [37].

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